

Vironostika® HIV-1 Plus O Microelisa System



Store between 2-8°C.

For *in vitro* diagnostic use.

INTENDED USE

The Vironostika® HIV-1 Plus O Microelisa System is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1), including Group O, in human specimens collected as serum, plasma, or dried blood spots. The Vironostika HIV-1 Plus O Microelisa System is intended for use as an aid in diagnosis of infection with HIV-1. It is not intended for use in screening blood donors.

SUMMARY AND EXPLANATION OF THE TEST

Published data indicate a strong correlation between the acquired immunodeficiency syndrome (AIDS) and a retrovirus referred to as Human Immunodeficiency Virus (HIV).^{1,2} Currently, two HIV serotypes, designated as HIV-1 and HIV-2, have been identified based on the results of serologic and molecular studies. Both HIV serotypes have been isolated from patients with AIDS and AIDS-related complex (ARC), as well as from apparently healthy individuals at high risk for AIDS.² Both viruses have the same morphology, lymphotropism,³ and modes of transmission.⁴ Since 1984, reports have indicated that HIV-1 can be isolated from a variety of tissues and body fluids of infected individuals.^{2,5} In 1990, a more divergent strain of HIV-1, which is known as Group O HIV-1, was isolated and characterized.⁶ Antibodies specific for group O HIV-1 are difficult to be detected with some HIV antibody detection systems unless these systems use whole viral lysate and/or contain group O specific epitopes as antigens.^{7,8} In addition, an increasing number of variants have been identified with HIV-1 serotype, some of which have arisen from genetic recombinations within a host having infection with multiple types of HIV-1. Such variants may be more difficult to be detected with ELISA systems that are not based on viral lysate.^{7,8} Worldwide distribution of HIV shows prevalence of the serotypes in different areas, with HIV-1 most widely distributed and Group O appearing primarily in West Central Africa.

Following infection with HIV, an individual rapidly (e.g. within 4 weeks) develops antibodies to viral proteins, a process known as seroconversion. After seroconversion, HIV specific antibodies can be readily detected in the blood specimen. A majority of patients who exhibit symptoms of AIDS or ARC have HIV specific antibodies in their blood. In addition, a significant proportion of apparently healthy individuals at increased risk for AIDS also contain HIV specific antibodies in their blood specimens. Due to close relation of HIV-1 and HIV-2, proteins of the two viruses, especially the core and polymerase proteins, result in serologic cross-reactivity.³

The Vironostika HIV-1 Plus O Microelisa System assay was designed to be highly sensitive for a spectrum of HIV-1 serotypes, including group O virus. As a result, nonspecific reactions may occasionally be seen in specimens from people who have prior pregnancy, blood transfusion, or exposure to human cells or media containing cultured HIV antigen.⁹ Because of these and other potential nonspecific reactions, specimens reactive with the Vironostika HIV-1 Plus O Microelisa System assay should be confirmed with a confirmatory test, e.g., Western Blot testing.

Reactive specimens upon initial testing with the Vironostika HIV-1 Plus O Microelisa System assay should be re-tested in duplicate. Reactivity in either or both of the duplicate tests indicates a potential for the presence of HIV-specific antibody. In individuals at increased risk of infection, such as homosexual men, hemophiliacs, or intravenous drug users, repeatedly reactive specimens are usually found to contain antibodies to HIV by additional, more specific tests. However, when the ELISA is used to screen populations with a low prevalence of HIV infections, nonspecific reactions may be more common than specific reaction. Information about prevalence of HIV infections in persons in various categories of risk, as well as clinical and public health guidelines, are available in each CDC publication of *Morbidity and Mortality Weekly Reports*. Although information about the degree of risk for HIV-1 infection and the degree of reactivity of the serum are of value in interpreting the test, a diagnosis should not be based only on this information. Therefore, it is appropriate to investigate repeatedly reactive specimens by additional, more specific tests, such as Western Blot, immunofluorescence, radioimmunoprecipitation, viral antigen based immunoassays, peptide ELISAs, or nucleic acid amplification assays.

PRINCIPLE OF THE TEST

This test uses HIV-1 antigens, which are coated onto the wells of microwell plates, for the detection of antibodies specific for HIV-1, including Group O. These antigens include inactivated, purified HIV-1 viral lysate proteins,¹⁰ purified viral envelope proteins, and a synthetic peptide with amino acid sequence corresponding to that of the transmembrane immunodominant domain of the HIV-1 Group O (ANT 70) isolate.⁶

Upon the addition of a diluted test specimen containing antibodies to HIV-1 or HIV-1 Group O to a microwell, immune complexes are formed through the interaction between anti-HIV in the specimen and HIV antigens coated on the microwell. Following incubation, the specimen is aspirated and the well is washed with buffer. Subsequently, anti-human immunoglobulin (goat) conjugated with horseradish peroxidase (HRP) is added which binds to the anti-HIV-antigen complex during a second incubation. Following a wash and incubation with ABTS (2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate]) substrate, a green color is produced. The enzyme reaction is stopped by the addition of a fluoride solution.¹¹ The amounts of antibodies to HIV present in the specimen are qualitatively proportional to color intensity.

REAGENTS

For *in vitro* diagnostic use.

Components in each Vironostika® HIV-1 Plus O Microelisa System

384 Tests	960 Tests	9,600 Tests	Component Description
4 stripholders	10 stripholders	100 stripholders	HIV-1 Plus O Microelisa Strips – Twelve per holder, each containing 8 HIV-1 lysate (inactivated) and synthetic HIV-1 Group O antigen-coated wells, contained in a foil pack (2 stripholders/pack) with silica gel desiccant.
1 bottle (120 ml)	2 bottles (120 ml)	20 bottles (120 ml)	Dilsim™ III – Liquid specimen diluent; contains bovine and caprine proteins, salt, surfactant, Chlorophenol Red as specimen addition indicator, and antimicrobial agents (contains 0.03% [w/v] bromonitrodioxane).
3 vials (0.5 ml)	6 vials (0.5 ml)	18 vials (0.5 ml)	Negative Calibrator Serum (Human) – Contains human serum and goat serum as stabilizer with 0.05% (w/v) bromonitrodioxane as preservative and Fast Green FCF as coloring agent; nonreactive to HBsAg, antibodies to HIV-1 or HCV.
1 vial (0.5 ml)	2 vials (0.5 ml)	6 vials (0.5 ml)	HIV-1 Positive Control Serum (Human) – Inactivated human serum containing protein stabilizers. Contains 0.05% (w/v) bromonitrodioxane as preservative and FD&C red dye no. 2 as coloring agent; reactive for antibodies to HIV-1 and nonreactive to HBsAg and antibodies to HCV.
1 vial (0.5 ml)	2 vials (0.5 ml)	6 vials (0.5 ml)	HIV-O Positive Control Serum (Human) – Inactivated human serum containing protein stabilizers. Contains 0.05% (w/v) bromonitrodioxane as preservative and Orange II as coloring agent; reactive for antibodies to HIV-1 Group O and nonreactive to HBsAg and antibodies to HCV.

2 vials	4 vials	38 vials	Peroxidase Conjugated Goat Anti-human Immunoglobulins (EnzAbody®) – Lyophilized with protein stabilizers and FD&C red dye no. 2.
1 bottle (120 ml)	2 bottles (120 ml)	16 bottles (120 ml)	Conjugate Diluent – Phosphate buffered saline containing protein stabilizers and 0.03% (w/v) bromonitrodioxane as preservative.
2 bottles (62 ml)	3 bottles (62 ml)	32 bottles (62 ml)	ABTS Substrate Solution – 2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate], containing hydrogen peroxide.
1 bottle (120 ml)	2 bottles (120 ml)	16 bottles (120 ml)	Stop Solution – Contains 0.28% sodium fluoride and 0.03% (w/v) bromonitrodioxane as preservative.
10 sheets	40 sheets	400 sheets	Plate sealers – Adhesive.
1 each	1 each	4 each	Clamp and rod – Closure for foil pack.

Note: The Wash Concentrate and DBS Elution Medium are provided separately and individually as accessories to the kit:

Accessory Product Number	Quantity	Accessory Description
250622	4 bottles (500 ml)	Wash Concentrate 50X – Contains 2.5% surfactant in phosphate buffered medium containing 0.05% (w/v) bromonitrodioxane.
250626	4 bottles (120 ml)	DBS Elution Medium – Liquid specimen diluent; contains bovine and caprine proteins, salt, surfactant, and anti-microbial agents (contains 0.03% [w/v] bromonitrodioxane).

Note: Dilsim III and DBS Elution Medium contain Dilsim, a patented diluent.

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

This test kit is intended for use with serum, plasma, or dried blood spots.

1. Caution: Handle all Vironostika® HIV-1 Plus O Microelisa System biological materials as though capable of transmitting infectious agents. The antigen used to coat the microelisa wells and the positive control sera have been inactivated; nevertheless, both should be handled as though they contain potentially infectious agents. Other components prepared from human serum or plasma have been tested using FDA-licensed tests and found to be nonreactive for the presence of HIV-1 antibody, HTLV-I/II antibody, hepatitis B surface antigen (HBsAg) and HCV antibody. However, as no test method can offer complete assurance that infectious agents are absent, all materials of human origin should be handled as though they contain infectious agents.
2. Do not pipet any of the materials by mouth. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
3. Do not perform the test in the presence of reactive vapors (e.g., from sodium hypochlorite, acids, alkalis, or aldehydes) or dust, because the enzymatic activity of the conjugate may be affected.
4. Use disposable gloves. Handle specimens and materials contacting specimens as potentially infectious biological materials in accordance with "Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and Bloodborne Pathogens in Health-Care Setting" (CDC, MMWR, June 24, 1988). Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations, lesions, or other breaks in the skin.

5. Immediately clean up any spillage of material potentially containing antigen or antibody with a 1:10 dilution of 5% sodium hypochlorite. Dispose of the cleaning material by an acceptable method.
6. Dispose of all specimens and materials used to perform the test according to local guidelines. For example:
 - a) Autoclave for 60 minutes at 121°C.
 - b) Incinerate disposable materials.
 - c) Mix liquid waste with 5% sodium hypochlorite solution so that the final concentration is approximately 0.5% sodium hypochlorite. Allow to stand at least 30 minutes before disposal.
7. The **Stop Solution** contains sodium fluoride. Avoid contact with skin. If contact is made, thoroughly wash area with water.

REAGENT PREPARATION

Prepare all reagents before beginning assay procedure. All reagents and specimens should be at room temperature (15-30°C) before beginning the assay procedure and can remain at room temperature during testing.

Wash Solution

1. Dilute the **Wash Concentrate** 1:50 with purified¹⁵ or NCCLS Type I¹⁶ water in a clean container. Prepare at least 50 ml of **Wash Solution** for each **HIV-1 Plus O Microelisa Strip**. Refer to preparation chart.

Preparation of Wash Solution		
Number of Microelisa Strips	Volume of Wash Concentrate (ml)	Volume of Purified Water (ml)
1-6	16	784
7-12	32	1568

Number of Microelisa Plates	Volume of Wash Concentrate (ml)	Volume of Purified Water (ml)
1	32	1568
2	64	3136
3	96	4704
4	128	6272
5	160	7840
6	192	9408
7	224	10976
8	256	12544
9	288	14112
10	320	15680

The total volume of Wash Solution does not include any additional volume required for an automated washer (priming, dead volume, etc.). Refer to the manufacturer's instructions for the microelisa plate washer.

2. Label the container "**Wash Solution**." Add 7 days to date of preparation and record date on container label, along with the statement "Use before (that date)." Store **Wash Solution** at room temperature.

EnzAbody

1. Pipet 50 ml **Conjugate Diluent** into 1 vial of **EnzAbody**. Mix by inverting several times. Avoid excessive foaming. Allow **EnzAbody** to rehydrate a minimum of 30 minutes before use. Mix again before using to ensure a homogeneous solution. All dye particles should be in solution prior to use.
2. Add 28 days to date of reconstitution and record date on vial label, along with the statement "Use before (that date)." Store prepared **EnzAbody** at 2-8°C.

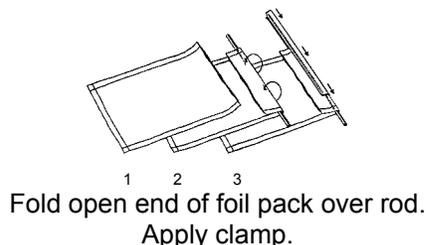
KIT STORAGE INSTRUCTIONS

Store kit reagents at 2-8°C when not in use. The expiration date printed on the kit indicates the date beyond which the product should not be used. Stability of kit reagents after reconstitution or dilution is listed in "REAGENT PREPARATION." Do not store frozen.

HIV-1 Plus O Microelisa Strips

The foil packs should be brought to room temperature (15-30°C) before opening to prevent condensation on the **HIV-1 Plus O Microelisa Strips**. After the airtight foil pack has been opened the Strips are stable for 4 weeks at 2-8°C if the foil pack is resealed with the clamp and rod or equivalent closure. The silica gel bag must not be removed.

Figure 1: Foil Pack Closure.



CHEMICAL OR PHYSICAL INDICATIONS OF INSTABILITY

Alterations in the physical appearance of test kit material may indicate instability or deterioration. The expiration date shown on component labels indicates the date beyond which product should not be used.

SPECIMEN COLLECTION, STORAGE AND SHIPMENT

Collection:

Serum or Plasma

No special preparation or fasting of the patient is necessary. Serum or plasma derived from heparin, citrate, or EDTA (ethylenediaminetetraacetate) as anticoagulants may be used. Serum or plasma separation tubes may be used. Serum or plasma heat inactivated at 56°C for 30 minutes may be used.

Dried Blood Spots

1. Collect newborn blood onto filter paper using the procedure described in the NCCLS publication *Blood Collection on Filter Paper for Neonatal Screening Programs*.¹²
2. Collect adult blood by one of the following methods:
 - a) **Direct Fingerstick:**^{12,13} Gently touch the filter paper (Schleicher and Schuell #903 or equivalent) to the drop of blood on the finger.
 - b) **Indirect Fingerstick:**^{12,13} Collect the blood in a capillary tube and express a drop onto the filter paper. Avoid painting or smearing the blood onto the filter paper with the capillary tube.
 - c) **Venipuncture:** Collect a blood sample in an appropriate tube containing anticoagulants (heparin, citrate, or EDTA). Transfer enough blood (approximately 100 to 150 µl) onto the filter paper to fill slightly beyond the printed edge of the circle.¹²

Notes:

With fingersticks, the first drop should be wiped away before collection since the first drop is most likely to contain excess tissue fluid.^{12,13}

The blood should soak completely into the filter paper. Prepare one or more spots for each patient. Blood should not be applied to both sides of the filter paper nor should clotted blood be smeared onto the filter paper.

Allow the specimens to dry in a horizontal position without touching any surface for at least three hours.¹² The filter paper may be allowed to dry at room temperature overnight.

3. Do not test dried blood specimens having any of the following characteristics:
 - Foreign substance contamination
 - Blood clots
 - Non-uniform saturation with blood

Storage:*Serum or Plasma*

Specimens should be free of microbial contamination and can be stored at 2-8°C for up to 7 days. For long-term storage, specimens should be frozen at -20°C or colder. Specimens repeatedly frozen and thawed more than five (5) times or those containing particulate matter may give erroneous results.

Dried Blood Spots

Dried blood spots may be stored refrigerated (2-8°C), or at room temperature (15-30°C) for 90 days as long as they are not exposed to elevated humidity (>50%). For long-term storage, dried blood spots may be frozen at -20°C or colder at <50% humidity. Although specimens exposed to humidity ≥50% and elevated temperature (37°C) for 14 days did not exhibit a detectable loss in reactivity, bioMérieux does not recommend routine storage of dried blood spots at elevated temperatures and humidity.

Shipment:

Specimens to be shipped must be packaged in compliance with federal regulations governing the transport of etiologic agents.

Dried Blood Spots

For shipment, dried blood spot specimens should be placed in a sealed container, such as a heavy-duty zippered bag with a desiccant. Exposure to greater than 50% relative humidity may adversely affect stability of the specimen.

VIRONOSTIKA® HIV-1 PLUS O MICROELISA TEST PROCEDURE**Materials provided**

HIV-1 Plus O Microelisa Strips

Dilsim III

Negative Calibrator Serum (Human)

HIV-1 Positive Control Serum (Human)

HIV-O Positive Control Serum (Human)

Peroxidase Conjugated Goat Anti-human Immunoglobulins (EnzAbody)

Conjugate Diluent

ABTS Substrate Solution

Stop Solution

Plate sealers

Additional materials required but not provided

Instruments/Equipment

Note: For any instrument, the manual provided by the manufacturer should be reviewed for additional information regarding the following:

1. Installation and special requirements.
2. Operation principles, instructions, precautions, and hazards.
3. Equipment calibration.
4. Manufacturer's specifications and performance capabilities.
5. Service and maintenance information.
6. Quality Control.

Automated diluter/dispenser system (minimum 10 μ l with 10% accuracy), test tubes, or equivalent
Aspiration/wash system

The aspiration/wash system must be capable of dispensing a minimum volume of 300 μ l, and capable of performing a minimum 30 second soak cycle. Aspirated waste must be contained in a closed system.

Adjustable multi-channel variable volume pipet system capable of delivering 50 – 300 μ l \pm 5%, and tips

Adjustable multi-channel variable volume pipet system capable of delivering 5 – 50 μ l \pm 5%, and tips

Micropipet(s) capable of delivering, 10 μ l \pm 10%, 500 μ l \pm 5%, and tips

Incubator

A dry incubator, heating block or equivalent, capable of maintaining 37 \pm 2°C.

Microelisa plate reader

Any microelisa reader capable of transmitting light at 405 nm \pm 5 nm with a linear absorbance range of 0 to 2.000.

Timer

Graduated cylinder, 50 ml

Reagents/Disposables

Wash Concentrate 50X (Product number 250622)

Clamp and rod (Product number 270880)

Purified water, USP¹⁵ or NCCLS Type I¹⁶ reagent water, or equivalent

Stripholder with uncoated wells (Product number 278849)

Absorbent paper

V-shaped disposable troughs or equivalent

Disposable gloves

Sodium hypochlorite solution (5%) or liquid bleach

Appropriate biohazard waste containers for materials potentially contaminated with infectious agents

Additional materials required but not provided for Dried Blood Spots

DBS Elution Medium (Product number 250626)

Cotton fiber filter paper (Schleicher and Schuell #903 or equivalent)

Lancets

1/4-inch or 1/8-inch hole punch

Plate shaker, rocker or rotator (for room temperature elution)

Zippered plastic bags, Bitran[®] (Saranex[®] Series S) plastic bags or equivalent

Uncoated microwell plates for elution (Corning Costar[®], Product number 9017 or equivalent)

Desiccant (if necessary)

Materials available from bioMérieux

Wash Concentrate 50X (Product number 250622)
DBS Elution Medium (Product number 250626)
Incubator
Microelisa washer
Microelisa reader
Stripholder with uncoated wells (Product number 278849)
12-channel pipet and tips
Micropipet and tips
Microprocessor-controlled diluter/dispenser

Procedural notes

1. **HIV-1 Plus O Microelisa Strips, EnzAbody, Negative Calibrator, and Positive Controls** used in an assay must be from the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (15-30°C) before testing begins. Return the reagents to 2-8°C after use.
2. **HIV-1 Plus O Microelisa Strips** of the microelisa plate are removable. Remove Strips not needed and replace with uncoated Strips. Store unused Strips as described in "KIT STORAGE INSTRUCTIONS." Before testing begins, inspect the microelisa stripholder and ensure that all wells are secure. Stripholders should be handled with care to ensure that no Strip is dislodged during testing. Strips may be numbered to ensure re-insertion should Strips become dislodged.
3. **HIV-1 Plus O Microelisa Strips** and plate sealers may be used only once.
4. Do not touch the top or bottom of strips, or the edge of wells with fingers or pipet tips.
5. All reagents and specimens must be mixed well before use. The Positive Controls and Negative Calibrator may be vortexed before pipetting. One of each Positive Control and three Negative Calibrator replicates must be run on each plate (stripholder). If more than one stripholder is processed, ensure that all specified incubation times are met.
6. This assay refers to reagents used to calculate the cutoff for assay results as "**Calibrators**." Instrument printouts and associated literature used in conjunction with this assay may refer to these reagents as "**Controls**." **This difference does not affect assay results.**
7. The **Vironostika HIV-1 Plus O Microelisa System** utilizes a **Negative Calibrator** to calculate the cutoff value (refer to note below regarding use of an external negative control).
Note: CLIA regulations require control reagents to be used according to 42 CFR 493. If a bloodborne pathogen test kit uses any of its manufacturer supplied reagents to serve as a **calibrator** function, i.e., either or both of the test kit controls (negative or positive) are used to calculate the assay cutoff, then CLIA regulations require that (an) additional "control" reagent(s) be included in each run. Such reagents may be procured or developed in-house. In any case, prior to placing the additional controls in routine use, each lot of such reagents should have: 1) a known dating period, i.e., validated stability (supplied by a control reagent manufacturer or validated by the user on in-house developed control reagent); and 2) known performance parameters, i.e., specifications for acceptance. Prior to implementation, additional control reagents should be qualified to minimize possible incompatibilities that may exist with particular test kits.
8. Do not allow the microelisa wells to dry once the assay has begun. Fill the wells with the next required reagent immediately after washing; if not possible, fill the wells within 10 minutes. The assay should be repeated if the wells can not be filled within 10 minutes after washing.
9. Inspect wells after wash steps. Remove any extraneous material on the bottom of any well that could interfere with absorbance reading.
10. All pipetting steps should be performed with the utmost care and accuracy. Cross-contamination between reagents will invalidate test results. Use micropipets for quantitative delivery of specimens and reagents. For the manual pipetting of controls, calibrator, and specimens, use individual, disposable specimen tips to prevent carryover of specimens. Avoid microbial or any other contamination of reagents.

11. If a specimen is inadvertently not added in this assay, e.g., a well is missed, the assay results for this specimen may be incorrectly interpreted as nonreactive. A missed specimen may be indicated by the lack of color shift in the **Dilsim III**.
12. Specimens with microbial contamination may not exhibit a color shift when diluted in **Dilsim III**.
13. Minimize opening the door of the incubator during the 37°C incubation time.
14. Avoid chemical contamination of reagents and equipment. Routine maintenance of the aspiration/wash system is strongly recommended to prevent carryover from highly reactive specimens to nonreactive specimens.
15. The aspiration/wash system should be flushed with copious amounts of water upon completion of the final wash of the assay. Use of water containing 0.01% (w/v) bromonitrodioxane will prevent microbial contamination from accumulating when equipment is not in use.
16. Manual plate washing should be validated before use. Use of an automated plate washer is recommended (refer to **Additional materials required but not provided** for automated washer requirements). Incomplete washing may adversely affect the test outcome.
17. Do not return leftover reagents to their original bottles.
18. Do not touch the bottom exterior surface of the microwells. Fingerprints or scratches may interfere with the reading of microwells.
19. Ensure that the Strips are leveled in the stripholder during the test procedure. If necessary, wipe the bottom of the Strips carefully with a soft, lint-free, absorbent tissue to remove any moisture, dust or debris before reading. If necessary, dried buffer may also be removed from the bottom of the Strips with a soft cloth dampened with water, then with a dry, soft, lint-free tissue before reading.
20. **Negative Calibrator** or **Positive Control** values that are not within the expected range (refer to Quality Control section) may indicate a problem with technique, product, or instrumentation.
21. All pipetting equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.
22. Bubbles in the Strip wells may cause inaccurate microwell readings. Care should be taken to ensure that no bubbles are present.
23. Use only properly calibrated equipment.
24. When punching disks from dried blood spot specimens, periodically remove accumulated paper fiber residue from the hole punch. This procedure reduces the possibility of cross-contamination from paper dust.
25. With dried blood spot specimens, the paper disk should appear almost white after elution (a faint brown color may remain in the disk).
26. The "Wash procedure" requires a soak cycle of at least 30-seconds.

Wash procedure

1. Incomplete washing will adversely affect the test outcome. Wash Solution must be at room temperature (15-30°C) before use.
2. Aspirate well contents into a waste flask. Then fill the wells completely without overflowing (approximately 0.3 ml) with Wash Solution. Aspirate and fill the wells a total of four times. Allow a minimum of 30-second soak period after each addition of Wash Solution.

Note: Failure to incorporate these soak periods into the wash procedure may result in increased numbers of falsely reactive specimens.

3. Ensure the Strips are completely aspirated after the final aspiration. If necessary, invert stripholder and tap firmly on absorbent paper to absorb excess Wash Solution. Care should be taken not to dislodge any Strips (gentle pressure applied to the sides of the stripholder during inversion will prevent dislodging of Strips).

Test procedure for serum or plasma specimens

Note: For Dried Blood Spots, see Test procedure for Dried Blood Spot (DBS) specimens below.

1. Fit stripholder with the required number of **HIV-1 Plus O Microelisa Strips**. If less than twelve Strips are needed, use uncoated strips to complete the plate when using a 96-well washer.
2. Prepare a 1:21 dilution of each serum or plasma test specimen, **Calibrator**, and **Controls**. Include three wells of Negative **Calibrator**, and one well each of **HIV-1 Positive Control** and **HIV-O Positive Control** on each run.

Caution: Use a clean tip for each specimen. Do not pipet specimen into an empty well without **Dilsim III**. Do not allow the microelisa wells to dry once the assay has begun.

- a) *Automated diluter/dispenser*: Pipet 10 µl of specimen, **Calibrator**, or **Control** with 200 µl **Dilsim III** into the designated microelisa well.
- b) *Premixed manual method*: Pipet 15 µl specimen, **Calibrator**, or **Control** into a clean test tube containing 300 µl **Dilsim III**. Mix well. Pipet 210 µl of the diluted specimen into the designated microelisa well.
- c) *Direct manual method*: Using a multichannel pipet, add 100 µl **Dilsim III** to each microelisa well. Pipet 10 µl specimen, **Calibrator**, or **Control** into the designated wells. Using a multichannel pipet and clean tips, add an additional 100 µl **Dilsim III** to each well and repeatedly aspirate and dispense to mix.

Note: The addition of a serum or plasma specimen to **Dilsim III** will cause the specimen addition indicator to turn the dilution to a lavender color.

3. Cover the Strips with adhesive plate sealers or equivalent. Within 60 minutes of specimen/control addition, incubate Strips at $37 \pm 2^\circ\text{C}$ for 60-70 minutes.
4. Wash each well four times with Wash Solution (refer to "Wash procedure") using a soak cycle of at least 30-seconds.
5. Pipet 150 µl of reconstituted **EnzAbody** working solution into each well.

Caution: Do not allow **EnzAbody** to contaminate **ABTS Substrate Solution**. If the same equipment is used to add both reagents, new disposable tips must be used.
6. Cover the Strips with adhesive plate sealers or equivalents. Incubate at $37 \pm 2^\circ\text{C}$ for 60 to 65 minutes.
7. Wash each well four times with Wash Solution (refer to "Wash procedure") using a soak cycle of at least 30-seconds.
8. Pipet 150 µl of **ABTS Substrate Solution** into each well. Do not mix or agitate. Do not cover the Strips.
9. Incubate at room temperature (15-30°C) for 10 to 13 minutes.
10. Stop the reaction by adding 150 µl of **Stop Solution** to each well (maintain the same sequence and time intervals used for **ABTS Substrate Solution** addition). Plates should be read within two hours.
11. Blank the microelisa reader on air (without stripholder and Strips) and read the absorbance of the solution in each well at 405 nm.

Test procedure for Dried Blood Spot (DBS) specimens

1. Prepare each dried blood spot specimen using one of the following methods. Ensure that the identity of each specimen is maintained.
 - a) *1/4-inch punch*: Punch one disk from each dried blood spot into a designated uncoated strip well. Pipet 150 µl of **DBS Elution Medium** into each well containing a punched dried blood spots and cover the Strips with a new adhesive plate sealer.
 - b) *1/8-inch punch*: Punch four disks from each dried blood spot into a designated uncoated strip well. Pipet 150 µl of **DBS Elution Medium** into each well containing punched dried blood spots and cover the Strips with a new adhesive plate sealer.

Note: To minimize carryover when retesting, the punch may be purged between spots by punching 2-3 clean areas of the filter paper. Remove disks and fibers from the punch by tapping lightly onto an absorbent paper for disposal.

2. Incubate at room temperature (15-30°C) for 60-90 minutes with agitation (400-600 rpm) or overnight (14-22 hours) at 2-8°C.
3. After incubation, fit a stripholder with the required number of **HIV-1 Plus O Microelisa Strips**. If less than twelve Strips are needed, use uncoated strips to complete the plate when using a 96-well washer.
4. Pipet 125 µl **Dilsim III** into each well of the **HIV-1 Plus O Microelisa Strip** that will be used for a dried blood spot specimen.
5. Before addition of DBS eluates to the test plate, mix eluates thoroughly using a plate shaker, or by repeatedly aspirating and dispensing contents with a pipet using a clean tip for each specimen. Transfer 25 µl of each eluate into the **HIV-1 Plus O Microelisa Strip** well containing **Dilsim III** and mix again.
6. Cover the elution plate containing the remaining dried blood spot eluate with a new plate sealer. Dried blood spot elutions may be stored at 2-8°C for 5 days. For long term storage, eluates may be frozen in microtubes or equivalent at -20°C or colder.
7. Prepare and pipet **Negative Calibrator**, **HIV-1 Positive Control** and **HIV-O Positive Control** as described in Step 2 under "Test procedure for serum or plasma specimens."
8. Cover the Strips with adhesive plate sealers or equivalent. Within 60 minutes of specimen/control addition, incubate Strips at $37 \pm 2^\circ\text{C}$ for 120-130 minutes.
9. Wash each well four times with Wash Solution (refer to "Wash procedure") using a minimum 30-second soak cycle.
10. Pipet 150 µl of reconstituted **EnzAbody** working solution into each well.
Caution: Do not allow **EnzAbody** to contaminate **ABTS Substrate Solution**. If the same equipment is used to add both reagents, new disposable tips must be used.
11. Cover the Strips with adhesive plate sealers or equivalent. Incubate at $37 \pm 2^\circ\text{C}$ for 60 to 65 minutes.
12. Wash each well four times with Wash Solution (refer to "Wash procedure") using a soak cycle of at least 30-seconds.
13. Pipet 150 µl of **ABTS Substrate Solution** into each well. Do not mix or agitate. Do not cover the Strips.
14. Incubate at room temperature (15-30°C) for 10 to 13 minutes.
15. Stop reaction by adding 150 µl of **Stop Solution** to each well (maintain the same sequence and time intervals used for **ABTS Substrate Solution** addition). Plates should be read within two hours.
16. Blank the microelisa reader on air (without stripholder and Strips) and read the absorbance of the solution in each well at 405 nm.

QUALITY CONTROL

Qualification of Negative Calibrator (NC) values:

The absorbances of each NC must be greater than or equal to 0.080 and less than or equal to 0.400. Eliminate outliers and calculate the NC mean (NCX). Absorbance of NC must be less than or equal to 1.5 multiplied by NCX and greater than or equal to 0.5 multiplied by NCX. If two or more values are outside range, the run is invalid and must be repeated.

Qualification of HIV-1 (PC₁) and HIV-O (PC₀) Positive Control values:

The absorbance values of the PC₁ and PC₀ must be greater than or equal to 0.700. If either the PC₁ or the PC₀ absorbance value is below this limit, the run is invalid and should be repeated.

TEST VALIDITY

The following criteria must be met in order for the test run to be considered valid:

1. The PC₁, PC₀, and NCs are qualified.
2. PC₁ - NCX ≥ 0.500.
3. PC₀ - NCX ≥ 0.500.

If any of these criteria are not met, technique may be suspect and the run must be repeated.

CALCULATION OF CUTOFF VALUE

Calculate the cutoff value (COV) as follows:

$$\text{COV} = \text{NCX} + 0.270$$

RESULTS

Calculations

Calculations must be made separately for each strip holder. Results are calculated and analyzed the same for serum, plasma, or dried blood spot specimens.

A test specimen is nonreactive if specimen absorbance is less than the cutoff value.

A test specimen is reactive if specimen absorbance is greater than or equal to the cutoff value.

Sample Calculations

Absorbance (example)

NC = 0.175, 0.195, 0.225

NCX = 0.198

PC₁ = 1.469

PC₀ = 1.111

Acceptance Criteria

Eliminate any calibrator or control absorbance values not meeting the following criteria:

$0.080 \leq \text{NC} \leq 0.400$

$\text{NC} \leq 1.5 (\text{NCX})$ or 0.297

$\text{NC} \geq 0.5 (\text{NCX})$ or 0.099

$\text{PC}_1 \geq 0.700$

$\text{PC}_0 \geq 0.700$

For the above example absorbances, none were eliminated.

Ensure that the difference between each positive control and NCX is acceptable.

$\text{PC}_1 - \text{NCX} \geq 0.500$

$1.469 - 0.198 = 1.271$ Pass

$\text{PC}_0 - \text{NCX} \geq 0.500$

$1.111 - 0.198 = 0.913$ Pass

The differences between each of the positive controls and the NCX are within acceptable limits for this example.

Calculate Cutoff Value

$\text{COV} = \text{NCX} + 0.270$

$\text{COV} = 0.198 + 0.270 = 0.468$

INTERPRETATION OF RESULTS

1. Specimens with absorbance values less than the cutoff value are considered nonreactive by the **Vironostika HIV-1 Plus O Microelisa System** criteria and may be considered negative for antibodies to HIV-1. No further testing is required.
2. Specimens with absorbance values greater than or equal to the cutoff value are considered initially reactive by the **Vironostika HIV-1 Plus O Microelisa System** criteria but before interpretation, the

specimen should be retested in duplicate. If either duplicate retest is reactive, the specimen is considered repeatedly reactive.

3. Initially reactive specimens that do not react in both of the duplicate repeat tests are considered negative for antibodies to HIV-1.
4. If the specimen is repeatedly reactive, the probability that antibodies to HIV are present is high, especially in specimens obtained from subjects at increased risk for HIV infection.¹⁴ In addition, persons who have participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. In most settings it is appropriate to investigate repeatedly reactive specimens by additional, more specific tests. Specimens found repeatedly reactive by ELISA and positive by additional, more specific tests are considered positive for antibodies to HIV-1. Clinical correlation is indicated with appropriate counseling, medical evaluation and possibly additional testing to decide whether a diagnosis of HIV infection is accurate. Interpretation of results of specimens found repeatedly reactive by ELISA and negative by additional, more specific tests is unclear; further clarification may be obtained by testing another specimen obtained three to six months later.

PERFORMANCE CHARACTERISTICS OF THE TEST

Reproducibility

Replicates of HIV-1 antibody positive serum, plasma, and dried blood spot specimens with various degrees of reactivity, negative specimens, and kit controls were tested at multiple sites (n=3), using multiple kit lots (n=3) and multiple technicians (n=3) on multiple days (n=4). Total, inter-assay, and intra-assay precision is reported in table 1 below. The total coefficient of variation (CV) for specimens 1-4 ranged from 11.0 to 22.4%.

Table 1: Assay Reproducibility

Specimen Type	ID	N	Mean	Total		Inter-assay		Intra-assay	
				SD	CV (%)	SD	CV (%)	SD	CV (%)
Serum or Plasma	1	72	2.78	0.561	20.2	0.533	19.2	0.185	6.7
	2	72	2.67	0.549	20.6	0.472	17.7	0.287	10.8
	3	72	5.48	0.602	11.0	0.538	9.8	0.278	5.1
	4	72	4.77	0.797	16.7	0.711	14.9	0.369	7.7
	NC	108	0.33	0.031	9.4				
	PC 1	36	4.78	0.732	15.3				
	PC O	36	4.04	0.883	21.8				
Dried Blood Spots	1	72	1.57	0.272	17.3	0.240	15.3	0.131	8.4
	2	72	1.86	0.416	22.4	0.347	18.7	0.232	12.5
	3	72	3.70	0.782	21.1	0.678	18.3	0.398	10.8
	4	72	3.60	0.676	18.8	0.611	16.9	0.299	8.3
	NC	108	0.34	0.036	10.7				
	PC 1	36	5.38	0.502	9.3				
	PC O	36	4.25	0.674	15.9				

ID	Specimen Identification
N	Number of Replicates
Mean	Mean Signal to Cutoff Ratio (SCR)
SD	Standard Deviation of SCR
CV	Coefficient of Variation of SCR
NC	Negative Control
PC 1	HIV-1 Positive Control
PC O	HIV-1 Group O Positive Control

Specificity

The specificity of the Vironostika HIV-1 Plus O Microelisa System was assessed by testing 6,032 serum/plasma specimens and 3,031 dried blood spot specimens collected from three low risk populations, including voluntary blood donors, insurance applicants and Planned Parenthood clinic patients. The licensed Vironostika HIV-1 Microelisa System was used for comparison. All specimens that were repeatedly reactive with either test were further tested with an FDA licensed Western blot assay.

Of the 6,032 serum/plasma specimens tested, thirteen (13) were repeatedly reactive with the Vironostika HIV-1 Plus O Microelisa System and subsequently confirmed HIV-1 antibody positive with a Western blot assay. In contrast, only 12 of the 13 positive specimens were reactive with the comparative test. As summarized in table 2, the specificity for the Vironostika HIV-1 Plus O Microelisa System in this study was estimated to be 100% (6,019/6,019), with a 95% confidence interval of 99.94 - 100%.

Of the 3,031 dried blood spot specimens, thirteen (13) were repeatedly reactive with the Vironostika HIV-1 Plus O Microelisa System. These repeatedly reactive specimens matched those of serum specimens, which were confirmed positive with a Western blot assay. The comparative test again detected only 12 of the 13 positive specimens. The specificity for the Vironostika HIV-1 Plus O Microelisa System was estimated in this study to be 100% (3,018/3018), with a 95% confidence interval of 99.88 - 100% for dried blood spots.

Table 2: Estimated Specificity in Low-Risk Populations

		Number tested	Non-reactive	Initially Reactive	Repeatedly Reactive	Western Blot Positive
Serum or Plasma	Population 1	1,500	1,500	0	0	N/A
	Population 2	3,012	3,012	0	0	N/A
	Population 3	1,520	1,506	14	13	13
	Total	6,032	6,018	14	13	13
Dried Blood Spot	Population 1	0	N/A	N/A	N/A	N/A
	Population 2	1,511	1,511	0	0	N/A
	Population 3	1,520	1,506	14	13	13
	Total	3,031	3,017	14	13	13

N/A Not applicable

The estimation of specificity is as follows:

$$\frac{(\text{Number screened} - \text{Number repeatedly reactive})}{(\text{Number screened} - \text{Number Western blot test positive})} \times 100$$

Sensitivity

The clinical sensitivity of the Vironostika HIV-1 Plus O Microelisa System was evaluated by testing matched serum/plasma specimens and dried blood spot specimens collected from 1,010 HIV-1 infected individuals with various CD4+ counts. These specimens were collected from six sites. Of the 1,010 dried blood spot specimens, 904 (90%) were collected directly as dried blood spots while 106 (10%) were collected with an indirect technique (100 µl of anticoagulated blood spotted onto filter paper).

As summarized in table 3, all serum/plasma specimens and dried blood spot specimens were repeatedly reactive with the Vironostika HIV-1 Plus O Microelisa System. Therefore, the sensitivity for both specimen types in this study was 100% (95% CI: 99.64-100%).

Table 3: Estimation of Clinical Sensitivity

Specimen Type	CD4+ Stratum	Number of specimens	Number of Initially Reactive	Number of Repeatedly Reactive
Serum or Plasma	<200	250	250	250
	200-499	385	385	385
	>499	375	375	375
	Total	1,010	1,010	1,010
Dried Blood Spots	<200	250	250	250
	200-499	385	385	385
	>499	375	375	375
	Total	1,010	1,010	1,010

High-risk populations

To assess the performance of the test with specimens collected from high-risk populations, fifteen hundred and fourteen (1,514) specimens were collected from four high-risk populations, which included prison inmates, STD (sexually transmitted diseases) clinic patients, inner city hospital emergency room patients, and HIV-1 outreach clinic patients. In addition, seven hundred and fifty (750) dried blood spot specimens were also collected from two of the study populations.

Serum/plasma specimens were tested with both the licensed Vironostika HIV-1 Microelisa System for comparison (comparative test) and the Vironostika HIV-1 Plus O Microelisa System. If repeatedly reactive with either or both tests, the specimens were further tested with a Western blot assay for confirmation. Dried blood spot specimens were tested only with the Vironostika HIV-1 Plus O. Table 4 lists the test results for the Vironostika HIV-1 Plus O Microelisa System.

Table 4: High-risk Populations

Specimen Type	Population	Number of specimens	Number of Initially Reactive	Number of Repeatedly Reactive	Western Blot Positive
Serum or Plasma	1	251	16	14	8
	2	513	13	13	13
	3	500	73	68	68
	4	250	28	27	27
	Total	1,514	130	122	116*
Dried Blood Spots	1	0	N/A	N/A	N/A
	2	0	N/A	N/A	N/A
	3	500	68	68	68
	4	250	27	27	27
	Total	750	95	95	95

*Three repeatedly reactive specimens in population 1 were indeterminate with the Western blot assay due to the presence of only a gp160, a gp120 or a 70 kDa band. The other 3 specimens were not tested by Western blot.

N/A Not applicable

Table 5 shows a summary of the agreement of test results obtained with the Vironostika HIV-1 Plus O and the licensed test with specimens from high risk populations. The 6 specimens that were repeatedly reactive (RR) with the Vironostika HIV-1 Plus O assay but non-reactive (NR) with the licensed test were considered to be false positive on the Vironostika HIV-1 Plus O assay. Therefore, the specificity of the Vironostika HIV-1 Plus O assay in this study of high risk populations was calculated to be $1392/1398 = 99.57\%$ (95% CI = 99.07% – 99.84%).

Table 5: Comparison of the Vironostika HIV-1 Plus O Assay with the licensed Vironostika HIV-1 Microelisa System in testing Specimens from High Risk Populations

Vironostika HIV-1 Plus O	Licensed Test		Total
	RR	NR	
RR	116	6*	122
NR	0	1392	1392
Total	116	1398	1514

*Western blot results were indeterminate for 3 of these specimens. The other 3 specimens were not tested by Western blot.

Seroconversion Panel Testing

Twelve (12) seroconversion panels were tested in triplicate with the Vironostika HIV-1 Plus O Microelisa System and the licensed Vironostika HIV-1 Microelisa System. Due to insufficient amounts of samples, only 10 of the 12 panels were also tested as DBS specimens.

For serum specimens, the Vironostika HIV-1 Plus O Microelisa System detects the reactivity earlier than the comparative test in all 12 panels.

Table 6: Seroconversion Panel Testing

Panel ID	Sample Collection Days	First Reactive Bleed (Days from the First Bleed)			
		Serum		DBS ¹	
		Vironostika HIV-1 Plus O System	Comparative Test ²	Vironostika HIV-1 Plus O System	Comparative Test ²
924	8, 10, 26, 33, 35, 40	33	40	33	NR
927	0, 28, 33, 35, 40	33	40	35	40
931	9, 15, 28, 33, 35, 42	28	33	28	33
932	0, 3, 13, 27, 34, 50, 78, 163, 194	34	NR ³	78	NR
940	0, 7, 11, 15, 18, 22, 25, 29	15	22	18	25
071	1, 3, 17, 22, 28	17	22	22	28
111	1, 2, 8, 16, 20, 22, 27	8	16	NT ⁴	NT
241	1, 7, 9, 15, 17, 22, 24	15	22	17	24
321	1, 8, 12, 15, 21	15	21	15	21
341	1, 7, 10, 21, 23, 28	21	28	28	28
351	1, 8, 11, 15	15	NR	NT	NT
361	1, 3, 9, 11, 16, 18	18	NR	NR	NR

¹Dried Blood Spot specimens

²The licensed Vironostika HIV-1 Microelisa System was used

³None of the specimens in this panel was reactive

⁴Not tested due to limited sample volumes

Dilution Panel Testing

Ten (10) serum specimens representing various HIV-1 group M clades and HIV-1 group O were used to prepare 10 serially diluted panels, which were then tested with the Vironostika HIV-1 Plus O Microelisa System and the licensed Vironostika HIV-1 Microelisa System. Each specimen was tested in duplicate with each of the three kit lots. The first dilutions that gave one or more non-reactive test result in each panel are presented in table 7. Highly diluted serum samples remained reactive with the test for all panels including the two HIV-1 group O panels. The Vironostika HIV-1 Plus O Microelisa System exhibited higher analytical sensitivity with the dilutional panels for both HIV-1 group M clades and HIV-1 group O specimens compared to the comparative test.

Table 7: First dilutions with non-reactive test results for each dilution series

Sample ID	Clade Type	Serum	
		Vironostika HIV-1 Plus O System	Comparative Test ¹
5805	HIV-1 Clade B	1:1,920	1:240
H629	HIV-1 Clade B	1:32,000	1:8,000
MD-O	HIV-1 Group O	1:25,600	1:400
302-1	HIV-1 Group O	1:1,600	NR ²
301-42	HIV-1 Clade A	1:12,800	1:1,600
302-18	HIV-1 Clade C	1:6,000	1:1,500
301-24	HIV-1 Clade D	1:48,000	1:3,000
302-23	HIV-1 Clade E	1:24,000	1:1,500
302-28	HIV-1 Clade F	1:12,800	1:1,600
302-17	HIV-1 Clade D	1:4,000	1:250

¹The licensed Vironostika HIV-1 Microelisa System

²Non-reactive

Detection of HIV-1 Group M Clades

Seventy-two (72) specimens representing infections with various clades of HIV-1 group M viruses were tested in duplicate with the Vironostika HIV-1 Plus O Microelisa system. Since there were limited amounts for most specimens, all specimens were diluted by 100 to 10,000 times prior to testing. Table 8 shows the test results for serum/plasma specimens. All specimens were repeatedly reactive with the assay, despite the dilution factors.

Table 8: Detection of HIV-1 Group M Clades

Clade	Number of Specimens	Sample Dilution Factor	Number of Repeatedly Reactive	Number of Non-reactive
A	10	100 to 10,000	10	0
B	12	100 to 10,000	12	0
B/D	1	100	1	0
C	10	100 to 10,000	10	0
C/E	1	1,000	1	0
D	10	100 to 10,000	10	0
E	7	100 to 5,000	7	0
E/A	3	1,000	3	0
E/C	1	10,000	1	0
E/F	1	100	1	0
F	10	100 to 10,000	10	0
G	4	1,000 to 5,000	4	0
H	2	1,000	2	0
Total	72		72	0

Detection of HIV-1 Group O Specimens

Archived serum specimens from eleven (11) HIV-1 Group O infected individuals were tested after dilution. These specimens were tested in duplicate with both the Vironostika HIV-1 Plus O Microelisa System and the licensed Vironostika HIV-1 Microelisa System. As shown in table 9, all eleven (11) specimens were repeatedly reactive with the Vironostika HIV-1 Plus O Microelisa System, while only 7 of the 11 were reactive with the comparative test. For all 11 specimens, the Signal to Cutoff Ratio (SCRs) were significantly higher with the Vironostika HIV-1 Plus O as compared to the licensed Vironostika HIV-1 based on the Wilcoxon Signed Rank test.

Table 9: Detection of HIV-1 Group O Specimens

Specimen ID	Sample Dilution	Vironostika HIV-1 Plus O System	Comparative Test ¹
		Mean SCR ²	Mean SCR
1	1:100	6.1	2.0
2	1:50	4.0	0.4
3	1:50	7.4	1.6
4	1:50	5.6	1.1
5	1:50	7.7	2.0
6	1:50	7.7	7.2
7	1:50	5.3	0.5
8	1:50	7.6	2.2
9	1:50	5.9	0.4
10	1:50	3.9	0.4
11	1:50	7.6	4.5
Total Repeatedly Reactive		11/11	7/11

¹The licensed Vironostika HIV-1 Microelisa System

²SCR = Signal to Cutoff Ratio

Detection of HIV-2 Positive Specimens

Twenty (20) HIV-2 positive serum/plasma specimens were diluted and tested in duplicate with the Vironostika HIV-1 Plus O Microelisa System. All HIV-2 specimens were repeatedly reactive with the Vironostika HIV-1 Plus O Microelisa System. The test results are listed below in table 10.

Table 10: Detection of HIV-2 Specimens

Specimen ID	Sample Dilution	Mean SCR*
1	1:10	3.3
2	1:10	5.8
3	1:500	1.5
4	1:10	4.3
5	1:50	1.7
6	1:10	5.5
7	1:10	2.5
8	1:10	5.0
9	1:100	3.7
10	1:1,000	1.8
11	1:200	1.3
12	1:200	1.4
13	1:10	1.9
14	1:50	1.3
15	1:1,000	4.9
16	1:1,000	3.6
17	1:50	1.7
18	1:1,000	2.7
19	1:10	2.0
20	1:10	1.8

*Specimens with SCR equal to or greater than 1.0 are considered reactive with the test.

Reactivity with Potentially Interfering Substances or Medical Conditions

Serum or plasma specimens (S/P)

Samples were collected from individuals with medical conditions that may cause nonspecific assay reactivity. These specimens were tested using the Vironostika HIV-1 Plus O Microelisa System. As shown in table 11, all but one specimens (184/185) were non reactive with the test. The one specimen weakly reactive with the test was from an individual with elevated bilirubin. Further analysis of this specimen with a Western blot assay showed the presence of antibodies weakly reactive with gp160. Therefore, elevated bilirubin was unlikely the cause for the reactivity of the specimen. Rather, the presence of nonspecific antibody in the sample contributed to the weak reactivity.

In a second study, the 184 non-reactive samples were spiked with a small volume of HIV-1 positive sample and tested with the system. All specimens were reactive with the Vironostika HIV-1 Plus O Microelisa System. The medical conditions listed in table 11 did not affect the reactivity of specimen spiked with HIV-1 seropositive sample.

Dried blood spot specimens (DBS)

A similar study was also performed using dried blood spot specimens. As shown in table 11, all 184 specimens were non-reactive with the Vironostika HIV-1 Plus O Microelisa System. When spiked with a small volume of HIV-1 seropositive sample, all 184 specimens were reactive with the system. No interference was observed with dried blood spot specimens.

Table 11: Reactivity of Specimens from Individuals with Potentially Interfering Substances or with Medical Conditions

Specimen type	Number of specimens		Neat Specimens				Spiked Specimens			
			No. of Reactive		No. of non-reactive		No. of Reactive		No. of non-reactive	
	S/P	DBS	S/P	DBS	S/P	DBS	S/P	DBS	S/P	DBS
Antinuclear antibody positive	10	10	0	0	10	10	10	10	0	0
CMV antibody positive	10	10	0	0	10	10	10	10	0	0
EBV antibody positive	10	10	0	0	10	10	10	10	0	0
Rubella antibody positive	9	9	0	0	9	9	9	9	0	0
Elevated bilirubin ¹	10	10	1	0	9	10	9	9	0	0
Hemolysed specimens	10	10	0	0	10	10	10	10	0	0
HSV-1 or HSV-2 antibody positive	10	10	0	0	10	10	10	10	0	0
HTLV-I or HTLV-II antibody positive	10	10	0	0	10	10	10	10	0	0
Lipemic ²	10	10	0	0	10	10	10	10	0	0
Multiple transfusion	10	10	0	0	10	10	10	10	0	0
Multiparous females	10	10	0	0	10	10	10	10	0	0
Rhematoid factor positive	10	10	0	0	10	10	10	10	0	0
SLE positive	10	10	0	0	10	10	10	10	0	0
Syphilis antibody positive	10	10	0	0	10	10	10	10	0	0
<i>Toxaplasmosis gondii</i> positive	7	7	0	0	7	7	7	7	0	0
HBV antigen positive	10	10	0	0	10	10	10	10	0	0
HCV antibody positive	10	10	0	0	10	10	10	10	0	0
Hypergammaglobulinemia	9	8	0	0	9	8	9	8	0	0
Influenza vaccinated	10	10	0	0	10	10	10	10	0	0
Total	185	184	1	0	184	184	184	184	0	0

¹ >6.3 mg/dL

² >980 mg/dL

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